CHAPTER IV
DISCUSSION AND CONCLUSIONS

Systematic biochemical and histopathological studies carried out in patients with diagnosed chronic liver diseases including cirrhosis and hepatocellular carcinoma confirm the important pathogenetic role of commonly occurring accumulation of iron deposits and metabolic disturbance like fatty liver. In this study HF diet increased plasma triglyceride and cholesterol levels of the rats. Chitosan reduced a progressive increase of plasma triglyceride and cholesterol, possibly by interfering digestion and absorption of dietary lipids via electrostatic interaction or/and trapping mechanism (Kanauchi et al., 1995; Mhurchu et al., 2004), as a result of inhibition of pancreatic lipase (Sumiyoshi and Kimura, 2006). Controversially, one study shows that chitosan had hypocholesterolemic effect in diabetic rats (Yao et al., 2008), and other studies show it did not change plasma lipid profiles (Bondiolotti et al., 2007). Previuos findings have shown that chitosan decreased levels of plasma total cholesterol and triglyceride of hypercholesterolemic rats (Hossain et al., 2007). Ausar and colleagues have indicated that chitosan (2%, *w*/*w*) increases high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in dyslipidemic plasma of type II diabetic patients, without changing levels of plasma triglyceride (Ausar et al., 2003). Fortunately, chitosan did not elevated levels of plasma ALT and AST of HF-fed mice (Sumiyoshi and Kimura, 2006) consistent with the result obtained from this study (as shown in **Table 3-9** and **Figure 3-15**).

The HF diet also increased liver triglyceride concentrations of the rats significantly; however, chitosan inhibited such progressive increase of the liver triglyceride. A previous study showed that chitosan significantly decreased liver cholesterol concentrations of the HF-fed Sprague-Dawley rats (Jennings et al., 1988). Chitosan can improve fatty liver and hyperlipidemia in HF-fed mice through inhibition of intestinal fat absorption (Han et al., 1999), and also suppress fat accumulation in the livers of HF-fed rats (Shigematsu et al., 2001). Moreover, Hossain and coworkers demonstrated that oral administration of shrimp (*Macrobracium rosenbergii*) chitosan increased levels of liver lipid peroxide in

hypercholesterolemic rats without affecting level of plasma ALT (Hossain et al., 2007).

In this work, liver ferritin concentrations were increased in HF-fed rats while chitosan inhibited a progressive increase of the liver ferritin concentrations. Elevated serum ferritin level associated with iron overload is proposed a major risk factor predicting nonalcoholic fatty liver disease (NAFLD) (Hsiao et al., 2004), and has been associated with steatohepatitis and fibrosis (Canbakan et al., 2007). We found that chitosan contributed to increase the liver iron concentration significantly of the rats being fed with both NF- and HF-diets. A previous report showed that chitosan did not change levels of blood hemoglobin and serum iron of the HF-fed rats (Jennings et al., 1988). Clearly, ferrocene-supplemented diet increased the liver iron concentration significantly in both WT and BKO thalassemic mice by means of enhancing intestinal absorption of the dietary iron. It has been recently reported that the BKO mice mimic β-thalassemia intermedia patients who have their blood hemoglobin level at 8.5-10 g/dl and become iron deficiency anemia. When they were challenged with high iron diet, their iron turnover rate; in particular duodenal absorption of dietary iron is accelerated. The antioxidant, iron-chelating GT extract was able to remove iron accumulation from their liver ($p < 0.05$), so was a reference oral iron chelator DFP. Recent studies have supported the ideas that EGCG and ECG in green tea use the galloyl group in their molecules to chelate iron (Srichairatanakool et al., 2006). Iron chelation of green tea catechin also improvsse iron overload and oxidative stress in some symptoms and diseases (Mandel et al., 2006; Mascitelli et al., 2006). A current investigation has demonstrated that green tea was effective in lowing iron overload in iron-induced rats (Ounjaijean et al., 2007).

Peroxidative decomposition of cellular membrane lipids is postulated a mechanism of hepatocellular injury in parenchymal iron overload. Findings in this study have indicated that the rats fed with HF diet had higher liver malondialdehyde (MDA) concentrations than those fed with NF diet while chitosan abated a progressive increase of the liver MDA concentrations successfully. Lykkesfeldt et al have shown that levels of hepatic GSH and MDA were increased in the ferrocene-treated rats in response to increasing concentrations of iron in the liver (Lykkesfeldt et

al., 2007). Iron can facilitate development of liver cirrhosis in carbon tetrachloride-induced mice (Arezzini et al., 2003). In this study, NF and HF diets significantly decreased levels of LIC of the rats, interestingly chitosan increased their LIC. Similarly, GTE and DFP effectively reduced the LIC in Fe diet-fed WT and BKO mice.

Green tea (GT) (*Camellia sinensis*) catechins, mainly EGCG, can lower plasma glucose and cholesterol concentrations (Yang and Koo, 2000; Zheng et al., 2004), attenuate cholestasis-induced liver fibrosis (Zhong et al., 2003), inhibit collagen production in hepatic stellate cells (HSC) (Nakamuta et al., 2005) and protect HepG2 cell injury (Lau et al., 2002). EGCG in GT regulates the structure and growth of HSCs and has therapeutic potential in the setting of liver fibrosis (Higashi et al., 2005). Treatment with ECGC protects the liver after ischemic/reperfusion possibly by reducing hepatic fat content, increasing hepatic energy status and functioning as a potent antioxidant (Fiorini et al., 2005). A current report recommends that consumption of green tea should reduce the risk of liver disease (Jin et al., 2008).

Chronic ROS production during obesity promotes developing of oxidative stress and appears by lipoperoxyl radical's formation in liver. GT catechins restore mitochondrial and microsome electron transport chain disorders and decrease intensity of peroxidation (Chanadiri et al., 2006). Green tea affects fat accumulation in the hepatocytes and protect against hepatic steatosis and disruption (Baltaziak et al., 2004). Result from Masson's trichrome stained-liver tissue has shown that GT catechins prevent and/or attenuate development of fibrosis in hepatitis (Abe et al., 2007).

HSC activation and hepatic fibrogenesis can be stimulated by oxidative stress. Glutathione (GSH) is the most important intracellular antioxidant which functions to counteract the ROS. EGCG attenuates oxidative stress in the HSC by scavenging the ROS and reducing lipid peroxidation. Insights into regulations of HSC activation may lead to new anti-fibrotic therapies, which may reduce morbidity and mortality in chronic liver-disease patients. Yumie and colleagues have demonstrated that GSH-derived antioxidant property of EGCG plays a critical role in anti-fibrogenic effect providing the prevention and treatment of liver fibrosis (Yumei et al., 2006). Long-term EGCG treatment attenuated the development of obesity and fatty liver whereas

short-term EGCG treatment reverses preexisting high-fat induced metabolic disorders in obese mice possibly by decreasing lipid absorption and inflammation (Bose et al., 2008).

In conclusion, chitosan can inhibit a progressive increase of triglyceride levels in plasma and liver of the rats significantly and reduce their liver ferritin concentrations, but did not affect levels of GSH in their livers. The compound tends to increase their liver iron and collagen concentrations slightly. GT catechins are effective in lowering iron deposition in livers of the iron-loaded WT and BKO mice effectively and significantly. It hardly changes GSH concentrations in livers of the iron-loaded WT and slightly decreases GSH concentrations in livers of BKO mice. As predicted, the two natural products can decrease levels of liver MDA significantly. Chitosan seems to not be harmful to livers of the treated rats. Collectively, benefical effects of green tea and chitosan including anti-oxidative, iron-chelating, free-radical scavenging, anti-lipid eproxidation, hepatic lipotropic properties would be a sharp bullet to hit or prevent occurrence or/and progression of liver fibrosis. Advantage of adjunctive or cocktail therapy over single therapy could give synergistic effects and eliminate adverse effects caused by chemical drug treatment. In prospective, the adjunctive therapy needs to be further investigated in β-thalassemia mice and patients with iron overload. Experimental designs have to be performed concisely and carefully. Efficacy of the adjunctive treatment should be evaluated and surrogate markers must be assigned, so that outcomes of the study will be applicable for the patients efficiently and successfully.